IMMUNOGENICITY OF HEPATITIS B VACCINE IN MULTI – TRANSFUSED THALASSEMIC PATIENTS WITH AND WITHOUT HEPATITIS C INFECTION: A COMPARATIVE STUDY WITH HEALTHY CONTROLS

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ABSTRACT

Hepatitis C virus (HCV) infection is highly prevalent in thalassemic patients, and this may decrease the serum antibody response to hepatitis B virus (HBV) vaccine. There is also some alteration of the immune system in multi-transfused thalassemic patients, as a consequence of iron overload. We investigated whether HCV infection may reduce the effectiveness of HBV vaccine in multi - transfused thalassemic patients. Subjects were cited and studied prospectively in three groups: group 1: 125 multi-transfused thalassemic patients with negative serum HCV antibody; group 2: 96 multi-transfused thalassemic patients with positive serum HCV antibody (ELISA II), in at least 2 different occasions; group 3: 100 healthy subjects. Matching was performed between three groups in sex, age and body mass index and subjects in all groups had negative serum HBsAg, anti-HBc and anti-HBs and received three 20 µgr/dose injections of recombinant HBV vaccine (Heberbiovac HB) in months 0, 1, 6. Anti-HBs titer was obtained one month after the last dose of vaccine and it was considered seroprotective if it was ≥ 10 IU/L. Seroprotection rate was 83.2% in group 1 and 80.2% in group 2 (*p*=0.74) and was 86% in healthy subjects, which didn't significantly differ with HCV positive and negative thalassemics (*p*>0.05). Moreover, the mean values of ALT among the responders and non-responder thalassemic patients were 55.5 ± 41.9 and 57.4 ± 48.5 U/L respectively(*p*=0.802). During vaccination periods, patients in all 3 groups did not show any significant adverse reactions. Our study shows that three standard doses of HBV vaccine are immunogenic and safe in multi-transfused thalassemic patients with or without HCV infection.
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INTRODUCTION

Hepatitis B vaccine has been shown to be highly immunogenic and efficacious in preventing clinical and subclinical infections in persons of different ages. Three

vaccinations with 10 μg or 20 μg recombinant HBsAg lead to seroprotection in 90 to 95% of young healthy individuals. [1-3] However, an increased risk of nonresponse has been associated with older age, male gender, chronic illness, immunodeficiency, smoking, obesity, and certain HLA types or haplotypes. [4-12] The immune response to HBV vaccine seems to be T-cell dependent and may be affected by conditions associated with impaired T-cell function. [12,13] HBV vaccination has been recommended for several well-recognized high-risk groups. One of these high-risk groups are patients with hepatitis C virus (HCV) infection. [14,15] Patients with HCV are at risk of additional liver damage when infected with other hepatotropic viruses, such as HBV. [16-18] Hepatitis C tends to evolve into chronic disease, more than any other viral liver disease. Thus, nearly 30% of cases finally will be chronic, and in about 60% of cases there will be abnormal liver enzymes for a long time. Seventy-three percent of patients will end in chronic active hepatitis, 51% in cirrhosis, and 5% in hepatic cancer in the long run. [19,20] Several factors contribute to development of the advanced compensated or decompensated forms of cirrhosis and hepatic cancer.[21] Host factors include certain HLA types, [22-24] positive viral RNA titers, [25] age at onset, [26-28] geographical factors, [29] natural course of the disease [30,31], and alcohol abuse. [32,33] The role of viral like co-infection with several species of HIVs, [34-37] and especially hepatitis B and C viruses has been suggested. [38-40] Co-infection of HBV and HCV frequently occurs. [39] It is thought that the reason for this high association is common risk factors for the two infections, such as transfusions, addiction, sexual transmission, and rarely intrauterine transmission. [15,18,41]

Unfortunately in most cases of HCV infection, there is no antigenic evidence of hepatitis B virus, expect for a HBV DNA marker. This (Anti-HCV+, HBsAg-, and HBV DNA+) is sometimes called hidden infection. [39] Moreover, when there are HBV and HCV infections simultaneously, response to interferon is less than HCV without any associated infection. Also, it is confirmed that HBV is an important risk factor for developing liver cancer in patients with chronic hepatitis C. [18,41] By attention to these issues, and with the presumption of avoiding additional liver injury, vaccination of all patients with HCV infection against HBV has been advocated. [15,18,40-43] In spite of the overall consequences of HCV infection its effects on the patients' immune system, are such that have made some believe that vaccination against HBV is less effective in these patients and some studies have shown that the response to vaccination against HBV

in patients with HCV infection was much less than what had been expected; [44-47] other recent studies however, did not confirm this and have shown acceptable immunization. [42-43, 48-50]

On the other hand, it is obvious that the vaccination of chronically transfused patients such as the transfusion dependent thalassemic subjects with HBV vaccine could contribute to a lessening of the risk for HBV transmission and is strongly necessary. [51] Moreover, many studies have concluded that the hepatitis B vaccine is safe, immunogenic and effective in thalassemics. [51-55] Although we well know about the effects of thalassemia and its resultant obligatory iron overload (in transfusion dependent thalassemic patients) on the immune system [56-58] but the effects of coincident thalassemia and HCV infection on the response to HBV vaccine, at least up to now, hasn't been the subject of any study.

So, in this study we have tried to overcome these shortcomings by comparison of responses to recombinant HBV vaccine in three groups; multi-transfused thalassemics without HCV infection, multi-transfused thalassemics with HCV infection, and healthy controls.

MATERIAL AND METHODS

The aim of this study was to determine the effect of HCV infection in multitransfused thalassemic patients on the efficacy of HBV vaccination, as reflected by serum anti-HBs levels. This study had been designed as a prospective trial by comparing the efficacy of HBV vaccine between three cohorts (see later) during a period of 12 months (from Mar. 2002 to Apr. 2003). A total of 321 individuals participated in our study, which were divided into three cohorts: multitransfused thalassemic patients without HCV infection, multitransfused thalassemic patients with HCV infection, and normal individuals. Thalassemic patients all were among those who received primary care services from the adult thalassemia center, Tehran, Iran, which provides medical care to more than 450 multitransfused adult thalassemic patients, and the normal participants have been selected from the healthy volunteer staffs and their relatives of that center and Imam Khomeini Hospital (the main affiliated hospital of the Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran). Ideally, the best sampling would be a random selection from three separate populations. But it was not possible to select samples randomly from this population. At first, a questionnaire was distributed to every individual, who was supposed to participate in the

Table 1. Characteristics of study participants.

	Multitransfused thalassemics without HCV infection n = 96	Multitransfused thalssemics with HCV infection n = 125	Healthy controls n = 100
Sex[+] (females)	46.4%	44.8%	46.0%
Age[+] (years) Mean ± SD Range	20.7 ± 3.1 12-29	20.2 ± 3.4 14-27	20.8 ± 2.9 16-27
Body mass index (kg/m²) Mean ± SD	19.5 ± 2.7	19.2 ± 2.2	19.3 ± 2.3
Splenectomy (yes)	57.6%	59.4%	-
Serum ALT[+] (U/L) Mean ± SD Range	37.6 ± 11.8 12-70	79.5 ± 55.7 10-290	19.8 ± 8.1 3-34

[+]Matching is performed between three groups.
[+]Differences within groups and between two thalassemic groups is significant (by ANOVA and independent samples t test, respectively): $p<0.0005$.

study. Then, those who met the primary including criteria (age < 35years, voluntariness, and consent) were referred to the laboratory for testing their anti-HBs and HCVAb titers. If anti-HBs testing was reported as negative, then the individual would be accepted into the study. One of these conditions accounted for exclusion criteria: history of HBV infection, anti-HBs > 10 IU/L, HBsAg+, anti-HBc+, anti-HIV+, and immunosuppressive agent use. Then, according to HCV titers and medical records, the accepted individuals in the previous phase were divided into three groups: HCV positive multitransfused thalassemic patients (by ELISA II in at least 2 different occasions), HCV negative multitransfused thalassemic patients, and normal individuals by matching in age and sex. The characteristics of each group of participants are shown in Table I. All the tests and lab measurements were implemented in two separate laboratories, the laboratory of the Adult Thalassemic Clinic and Gholhak laboratory. From then on, the first phase of clinical trial began. Phase I: All the participants were referred to the Iranian Pasteur Institute for three times standard vaccination at 0, 1, 6 months. The same persons did all the injections. All the recombinant vaccine doses were Heberbiovac R, of CGIB in Cuba. All doses were standard ones injected into deltoid muscles. Phase II: (examining responses to the vaccination): After one month, the anti-HBs titers were measured again in the same laboratory, by the same technician (for minimizing the inter-examiners variation), and with the same kit. Now, those whose anti-HBs titers were less than 10 IU/L were classified as non-responders and titers equal to or greater than 10 IU/L were classified as responders or seroprotective. Phase III: All the non-responders were given a booster (2 times standard) dose. As was pointed earlier, vaccination is recommended for all patients with HCV infection, [15,40-43] so participating in such a study during which all patients would be vaccinated with sufficient dose of HBV vaccine regardless of her/his HCV infection status, was not only harmful but even useful for all participants. However, the rational of the study was explained clearly to every participant and her /his written consent was obtained. Moreover, all services were free of charge, and nobody paid for any services being delivered. So, this trial was devised in accordance with the ethical principles outlined in the declaration of Helsinki, and laws and regulations of the Islamic Republic of Iran, accepted by regulatory affairs of Tehran University of Medical Sciences, Deputy of Research.

HBV vaccine efficacy in young healthy individuals is 90 to 95%. [1-3] Some studies however, have reported this efficacy in thalassemic patients without HCV infection as at least 80-100%. [51-55] But, HBV vaccine efficacy in multitransfused thalassemic patients with HCV infection had not been studied before, and apparently this was the first time that the effect of HCV infection in multitransfused thalassemic patients on HBV vaccine efficacy is being studied. By referring to these studies and considering 0.05 level of error for the first (α=0.05) and 20% for the second types of error (β=20%), we estimated a sample size about 85-90 and actually our sample size had exceeded these minimum values. Data entry, database management, and data analysis all were performed by SPSS 11.0 for Windows and differences in properties and mean values were tested with chi-square and independent samples t test (two-tailed), respectively. $p<0.05$ was considered significant.

Immunogenicity of Hepatitis B Vaccine in Thalassemics

Table II: Rates of seroprotection to vaccination with recombinant hepatitis B by anti-HBs response.

	anti-HBs<10 IU/L	10 IU/L ≤ anti-HBs≥100 IU/L	anti-HBs>100 IU/L
Healthy controls	14 (14.0%)	18 (18.0%)	68 (68.0%)
Thalassemics without HCV infection	21 (16.8%)	19 (15.2%)	85 (68.0%)
Thalassemics with HCV infection	19 (19.8%)	12 (12.5%)	65 (67.7%)
Total	54 (16.8%)	49 (15.3%)	218 (67.9%)

P value NS by chi-square test.

RESULTS

After administration of recombinant hepatitis B vaccine on a standard 0-1-6 month schedule, HBsAb titer was obtained one month after the final dose of vaccine and it was considered effective seroconversion (seroprotection) if it was more than 10 IU/L. The results of this assessment are shown in Table II and Figure 1. Seroconversion rate was 83.2% (104 of 125) in thalassemics without HCV infection and 80.2% (77 of 96) in thalasemics with HCV infection (*p*=0.74). Sevoconversion rate was about 86% in healthy subjects, which didn't significantly differ with HCV positive and negative thalassemics. During the vaccination period, patients in all 3 groups did not show any significant adverse reactions.
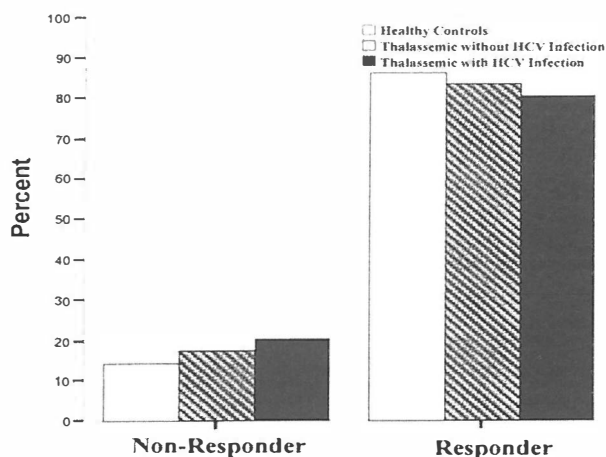

Fig 1. Rates of response to vaccination with recombinant hepatitis B vaccine, defined as seroprotection (anti-HBs=or>10 IU/L), in three groups of the study participants. Differences between these groups are not significant; *p*>0.05 by chi square.

By comparison between two groups of thalassemic patients, with and without splenectomy, we didn't find a considerable difference in seroprotection to HBV vaccine,

as seroprotection rates were 83.7% and 79.3%, respectively (Table III). Moreover, the mean values of ALT among the responders and non-responder thalassemic patients were 55.5 ± 41.9 and 57.4 ± 48.5 U/L respectively (*p*=0.802).

Table III. Rates of seroprotection to vaccination with recombinant hepatitis B in the studied thalassemic patients according to their splenectomy status.

	Seroprotection	
	YES (anti-HBs ≥ 10 IU/L)	**NO** (anti-HBs <10 IU/L)
With Splenectomy[†] (129)	108 (83.7%)	21 (16.3%)
Without Splenectomy[†] (92)	73 (79.3%)	19 (20.7%)
Total (221)	181 (81.9%)	40 (18.1%)

†*p* = 0.791 by chi-square test

DISCUSSION

The HBV vaccine is among the safest vaccines available and has been shown to be highly immunogenic and efficacious in preventing clinical and subclinical infection in persons of different ages. Three vaccinations with 10 to 20 µg recombinant HBsAg lead to seroprotection in 90 to 95% of young healthy individuals. [1-3] The immune response to HBV vaccine seems to be T-cell dependent and may be affected by conditions associated with impaired T-cell function. [12,13] The immune response to HBV vaccine in patients with chronic HCV infection has been evaluated in several studies. [42-49] Although improved seroprotection rates in HCV infected persons were reported in many studies, [42-43,48-50] decreased seroprotection of HBV vaccine has been shown by other studies. [44-47] Wiedmann et al. reported decreased

immunogenicity of 10μg of a recombinant HBV vaccine (HB-Vax) using 0-1-6 month schedule in patients with chronic HBV infection. The seroprotection rate (anti-HBs ≥ 10 mIU/mL) 3 months after the final dose was 69% in the 59 patients (mean age 42 years) and 91% in the control group of healthy adults. The possibility of latent HBV infection in non-seroprotective patients was excluded by negative testing for serum HBV-DNA. A high booster dose of 40 μg of a recombinant HBV vaccine in non-seroprotectives elicited a response in 12/15 (80%) individuals. [45] In the study of Leroy et al. after vaccination at 0-1-2 months with 20 μg recombinant HBV vaccine in 77 patients with chronic HCV infection and 231 healthy adults, only 63.6% of HCV infected patients were responders to vaccination (anti-HBs > 10 mIU/mL) compared with 93.9% of controls ($p<0.0001$). [46] Chalbicz et al., after vaccination of 48 HBV patients with HBV vaccine at 0-1-6 month schedule and assessment of their anti-HBs titers up to 18 months follow up, reported an overall seroprotection rate at 7 months of 72.9% in HBC patients, compared to 90.9% in the controls. At 18 months only 34.1% of the HCV patients had seroprotective titers, compared to 90% in the control group. [47] On the other hand in many other studies it has been shown that HBV recombinant vaccine is seroprotective in HBC infected patients. In one study conducted in Taiwan, in 26 patients with HCV infection as cases, and 35 normal individuals as controls, anti-HBS titers were measured regularly after administration of each dose of vaccine. Rates of responses between these two groups were similar; thus 30.8% and 17.1% after the first month, 61.5% and 60% after the second month, and 91.4% and 88.5% after the third month responded well respectively. There was not any change in HCV RNA titers in the patients' group during the same period, but a marked decrease in their serum ALT levels was detected. Accordingly, HBV vaccine had sufficient immunogenicity in patients with HCV infection, and could even cause liver enzymes' decrease. [42] Improved sero-protection rates (88.2-100%) were reported in studies using a higher dose (20 μg) of recombinant vaccine. [43,48-50] Many factors should be accounted for interpretation of these reports. For example, we know that the response to HBV vaccine is associated with age, sex, smoking, obesity, certain HLA types, [1-5,12-13] latent HBV infection, [39,59] and the extent of HCV load. [46]

What can be extracted from these studies is that vaccination of HCV patients with recombinant HBV vaccine is safe and doses do not exacerbate the underlying liver disease. Chronic infection may be associated with hyporesponsiveness to the vaccine and higher doses seem to be required to achieve optimal response. Post-vaccination anti-HBs responses should be measured and additional high dose boosters can be administered to overcome hyporesponsiveness. [59]

On the other hand, it is obvious that the vaccination of chronically transfused patients such as transfusion dependent thalassemic subjects with HBV vaccine could contribute to a lessening of the risk for HBV transmission and is strongly necessary. [51] Moreover many studies have concluded that the hepatitis B vaccine is safe, immunogenic and effective in thalassemics. These studies have reported at least 80-100% seroconversion in thalassemic patients without HCV infection. [51-55]

In this study we evaluated the effect of HCV infection in multitransfused thalassemic patients on the efficacy of HBV vaccination. For this purpose, we compared the anti-HBs responses to recombinant HBV vaccine in three cohorts; thalassemic patients without evidence of HCV infection, thalassemics with HCV infection, and healthy individuals. We showed that the seroprotection rates of HBV vaccine (defined as anti-HBs ≥ 10 mIU/mL, 1 month after final dose) were good and acceptable in these 3 groups. Our study shows that three standard doses of HBV vaccine is immunogenic and safe in multi-transfused thalassemic patients with or without HCV infection and we could not prove any effect for HCV infection on patients' immune system response to HBV vaccine.

On the base of these results, we suggest HBV vaccination of transfusion dependent thalassemic patients regardless of the presence of HCV infection. Obviously, evaluation of anti-HBs titers, at least annually, should be performed and booster doses must be administered in hyporesponsive subjects.

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